

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method for a long-term culture for more than 3 months of avian spermatogonial stem cells, which comprises the steps of:

(a) preparing an avian testis from an adult avian aged ~~[[up to]]~~ 2-70 weeks;

(b) isolating a population of testicular cells from said avian testis; ~~and~~

~~(c) culturing said avian spermatogonial stem cells in said population of testicular cells on a feeder cell layer in a medium containing a cell growth factor;~~

(c) culturing said population of testicular cells for about 5 to 10 days on plates in a medium containing a cell growth factor to form a colony of spermatogonial stem cells; and

(d) taking a colony of spermatogonial stem cells and culturing said avian spermatogonial stem cells for more than about 80 to 85 days on a feeder cell layer in a medium containing a cell growth factor;

wherein said step (b) is carried out by treating said avian testis with a mixture of collagenase and trypsin; said medium in steps (c) and (d) includes FBS (fetal bovine serum), avian serum, non-essential amino acids, Hepes buffer, and β -mercaptoethanol; and said feeder cell is avian gonadal stroma cell or testicular stroma cell.

2-6. (Canceled).

7. (Currently Amended) The method according to claim 1, wherein said cell growth factor is a growth factor selected from the group consisting of fibroblast growth factor, insulin-like growth factor-1, stem cell factor, ~~glial-derived~~ glia-derived neurotrophic factor and their combination.

8. (Original) The method according to claim 1, wherein said medium further comprises a differentiation inhibitory factor.

9. (Original) The method according to claim 8, wherein said differentiation inhibitory factor is leukemia inhibitory factor.

10. (Original) The method according to claim 1, wherein said medium comprises a supplement containing a mixture of fibroblast growth factor, insulin-like growth factor-1 and leukemia inhibitory factor.

11. (Original) The method according to claim 1, wherein said medium further comprises a serum and an antioxidant.

12. (Original) The method according to claim 1, wherein said culturing is carried out at about 37°C.

13. (Original) The method according to claim 1, wherein said avian species is a chicken, a quail, a turkey, a duck, a goose, a pheasant or a pigeon.

14. (Original) The method according to claim 1, wherein after step (c) said process further comprises the step of identifying the avian spermatogonial stem cells.

15. (Currently Amended) The method according to claim 14, wherein said identification is carried out by (i) PAS (Periodic Acid Schiff's) staining, (ii) STA (Solanum tubersum agglutinin) staining, (iii) a staining with $\alpha 6$ -integrin ~~$\alpha 6$ -integrin~~ antibody, (iv) a staining with $\beta 1$ -integrin ~~$\beta 1$ -integrin~~ antibody, (v) a staining with anti- SSEA-1 antibody, (vi) a staining with anti-SSEA-3 antibody, (vii) a staining with anti-SSEA-4 antibody, (viii) DBA (Dolichos biflorus agglutinin) staining or (ix) their combination.

16. (Withdrawn) A population of avian spermatogonial stem cells comprising avian cells expressing characteristics of a spermatogonial stem cell.

17. (Withdrawn and Currently Amended) The population of avian spermatogonial stem cells according to claim 16, wherein said characteristics of a spermatogonial stem cell is a positive reaction to (i) PAS (Periodic Acid Schiff's) staining, (ii) STA (Solanum tuberosum agglutinin) staining, (iii) a staining with $\alpha 6$ -integrin ~~$\alpha 6$ -integrin~~ antibody, (iv) a staining with $\beta 1$ -integrin ~~$\beta 1$ -integrin~~ antibody, (v) a staining with anti-SSEA-1 antibody, (vi) a staining with anti-SSEA-3 antibody, (vii) a staining with anti-SSEA-4 antibody, (viii) DBA (Dolichos biflorus agglutinin) staining or (ix) their combination.

18. (Withdrawn) The population of avian spermatogonial stem cells according to claim 16, wherein said population of avian spermatogonial stem cells is prepared in accordance with any one of claims 1-15.

19. (Withdrawn) A method for producing a transgenic ave, which comprises the steps of: (a) transferring a foreign gene to the population of avian spermatogonial stem cells according to any one of claims 16-18; (b) transplanting said population of avian spermatogonial stem cells into a testis of a recipient; and (c) producing a progeny from said recipient to produce the transgenic ave.

20. (Currently Amended) A method for a long-term culture for more than 3 months of avian spermatogonial stem cells, which comprises the steps of:

(a) preparing an avian testis from an avian aged 2-70 weeks;

(b) isolating a population of testicular cells from said avian testis; and

~~(c) culturing said avian spermatogonial stem cells in said population of testicular cells on a feeder cell layer in a medium containing a cell growth factor.~~

(c) culturing said population of testicular cells for about 5 to 10 days on plates in a medium containing a cell growth factor to form a colony of spermatogonial stem cells; and

(d) taking a colony of spermatogonial stem cells and culturing said avian spermatogonial stem cells for more than about 80 to 85 days on a feeder cell layer in a medium containing a cell growth factor;

wherein said step (b) is carried out by treating said avian testis with a mixture of collagenase and trypsin; said medium in steps (c) and (d) includes FBS (fetal bovine serum), avian serum, non-essential amino acids, Hepes buffer, and β -mercaptoethanol; and said feeder cell is avian gonadal stroma cell or testicular stroma cell.

21. (Previously Presented) The method of claim 20, wherein said avian is aged up to 20 weeks.

22. (Previously Presented) The method of claim 21, wherein said avian is aged 2-10 weeks.

23. (Previously Presented) The method of claim 20, wherein said avian is a chicken.

24. (Previously Presented) The method of claim 22, wherein said avian is a chicken.

25. (Currently Amended) A method for a long-term culture for more than 3 months of avian spermatogonial stem cells, which comprises the steps of:

(a) preparing an avian testis from an avian that is not in an embryonic stage;

(b) isolating a population of testicular cells from said avian testis; ~~and~~

~~(c) culturing said avian spermatogonial stem cells in said population of testicular cells on a feeder cell layer in a medium containing a cell growth factor.~~

(c) culturing said population of testicular cells for about 5 to 10 days on plates in a medium containing a cell growth factor to form a colony of spermatogonial stem cells;
and

(d) taking a colony of spermatogonial stem cells and culturing said avian spermatogonial

stem cells for more than about 80 to 85 days on a feeder cell layer in a medium containing a cell growth factor;

wherein said step (b) is carried out by treating said avian testis with a mixture of collagenase and trypsin; said medium in steps (c) and (d) includes FBS (fetal bovine serum), avian serum, non-essential amino acids, Hepes buffer, and β -mercaptoethanol; and said feeder cell is avian gonadal stroma cell or testicular stroma cell.